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Effects of 2,4-D with and without wiper-applied glyphosate on leafy spurge (*Euphorbia esula*) shoot, shoot regrowth, and root biomass

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Nomenclature: 2,4-D; glyphosate; leafy spurge, *Euphorbia esula* L.

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Competing interests

The authors declare no competing interests.

Abstract

Pot studies outdoors under natural environmental conditions were conducted to determine leafy spurge biomass reduction resulting from broadcast application of 2,4-D (2,244 g ae ha^{-1}) with and without wiper-applied glyphosate. Glyphosate (575 g ae L^{-1}) was applied at 0, 33, 50, and 75% diluted concentrate with a wiper 24 hrs after 2,4-D was broadcast applied. Injury estimates and shoot biomass did not differ between plants treated with 2,4-D-only or the addition of wiper-applied glyphosate 21 days after treatment. Shoot regrowth biomass of plants treated with 2,4-D-only was approximately 560% greater compared to nontreated plants three months after treatment. Plants treated with wiper-applied glyphosate had shoot regrowth biomass of less than 10% compared to the nontreated plants 3 months after treatment. Root biomass of 2,4-D-only treated plants (160% of nontreated plants) followed a similar pattern of shoot regrowth biomass. Root biomass of plants treated with wiper-applied glyphosate exhibited approximately 50% reductions compared to nontreated plants. The concentrations of glyphosate tested reduced all vegetative metrics equally; therefore, all labeled concentrations should be effective. The results of the experiment show that broadcast-applied 2,4-D is more effective at reducing leafy spurge biomass with the addition of wiper-applied glyphosate.

Keywords: wiper applicator, weed management

Introduction

Leafy spurge is a perennial broadleaf weed that inhabits various disturbed habitats, especially pasture and rangelands (Lym 1998). Management efforts need to be intensive and extensive to minimize spread since leafy spurge can reproduce both vegetatively and through seed production (Lym 1998; Morrow 1979). Therefore, simply ceasing seed production may not always be effective if the underground rhizomes remain viable (Jacobs et al. 2006; Wicks and Derscheid 1964). Few herbicides applied alone are effective on leafy spurge; effective herbicides include aminocyclopyrachlor (WSSA Group 4), imazapic (WSSA Group 2) and picloram (WSSA Group 4) (Lym 2014; Markle and Lym 2001). However, the effectiveness and sole reliance on these herbicides do not provide management longevity without the integration of other tactics (DiTomaso et al. 2017; Lym 1998). While nonchemical tactics are important for successful leafy spurge management, herbicides remain the most efficient tactic (DiTomaso 2000; Nelson and Lym 2003). 2,4-D (WSSA Group 4) is not effective alone in managing leafy spurge, but previous research has shown that the addition of 2,4-D in combination with other herbicides can additively increase the effectiveness (Al-Henaid et al. 1993; Gylling and Arnold 1985; Lym 2000).

Glyphosate (WSSA Group 9) is a nonselective herbicide that controls a wide spectrum of weed species (Duke and Powles 2008). Due to nonselectivity, this herbicide is rarely applied in pasture or rangeland due to the concern of suppressing or killing desirable grasses and forbs. Additionally, glyphosate applied alone is not recommended for leafy spurge management as the herbicide results in molecular changes that can also induce vegetative shoot and adventitious root growth when applied alone (Doğramacı et al. 2014; Doğramacı et al. 2016; Maxwell et al. 1987). Mixing glyphosate and 2,4-D can be effective for leafy spurge management, but desirable vegetation is injured or killed during broadcast sprays which can contribute to economic and ecosystem services losses (Gylling and Arnold 1985; Lym 2000). Wiper-applied herbicide applications are deployed to selectively manage weeds and allow for higher herbicide concentrations to be applied in grassland settings while reducing off-target injury to desirable vegetation (Grekul et al. 2005; Leif and Oelke 1990). Picloram has been applied with a wiper application to manage leafy spurge with success (Messersmith and Lym 1985). Wiper-applied glyphosate has also effectively managed Canada thistle (*Cirsium arvense* L.) in sensitive areas

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containing desirable vegetation (Krueger-Mangold et al. 2002). Since the desirable vegetation is nondamaged, plants can still be competitive with later emerging weeds (Lamb et al. 2024).

Despite the lack of efficacy on leafy spurge from broadcast glyphosate applications, the greater herbicide concentrations associated with a wiper application as a follow-up could increase the longevity of management. Since 2,4-D effectiveness is largely dependent on being mixed with another herbicide, glyphosate could be sequentially applied with a wiper to manage leafy spurge. Since 2,4-D and picloram have been applied extensively to manage leafy spurge, the inclusion of glyphosate could provide an additional management tool and disrupt previous selection pressure. Since 2,4-D and glyphosate are both readily absorbed and translocation throughout treated leafy spurge plants, sequential applications of both herbicides could increase leafy spurge control (Doğramacı et al. 2014; Maxwell et al. 1987) The objective of this research was to determine leafy spurge biomass reductions, including treated-shoots, and shoot and root regrowth resulting from broadcast application of 2,4-D alone and in combination with sequential wiper-applied glyphosate at various concentrations.

Materials and methods

Plant establishment

Leafy spurge plants were collected from a field site located at South Dakota State University in Brookings County, South Dakota (44.32567789590446 N, 96.77973203449149 W) in mid-June 2024. Plants were selected if yellow bracts were present and approximately 40 cm in height. Plants were carefully dug and transplanted into a 20 cm (6280 cm^3) pot containing an equal mixture of (Miracle-Gro, The Scotts Company LLC, Marysville, OH, USA), and field soil from the weed collection site (Marysland loam; a fine-loamy over sandy or sandy-skeletal, mixed, superactive, frigid Typic Calciaquolls). Plants were maintained outdoors under realized temperatures (average temperature: 27 C day/15 C night) and photoperiod (15 hr day/9 hr night) for the duration of the 4-month study. Pots were watered to saturation daily for two wks. Watering of pots to saturation thereafter occurred approximately every 2 d for the duration of the study.

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Broadcast and Wiper application

Treatments were arranged as a randomized complete block design with three replications. The experiment was conducted twice, where the plant collection and run initiation were separated by one week. After the plants were acclimated for 2 wks, plants were treated (excluding non-treated controls) with 2,4-D ester (Weedone LV4 Solventless [Nufarm, Cary, NC, USA; 480 g ae L^{-1}) applied at rate of 2,244 g ae ha^{-1}. The herbicide was applied using a CO_2-powered backpack sprayer at an output of 180 L ha^{-1} using Turbo TeeJet 8003 (TeeJet Technologies, Wheaton, IL, USA) nozzles 50 cm above the target plan. Leafy spurge plants were treated at approximately 40 cm in height and yellow bracts were present. The wiper-applied treatment occurred 24 hrs following the initial 2,4-D application. This delay was implemented to ensure the applied 2,4-D was absorbed into the plant and not transferred onto the wiper. The wiper applicator was positioned approximately halfway up the plant (20 cm) to simulate an application of herbicide above desirable vegetation growth height (Carlassare and Karsten 2002; Washburn and Seamans 2007). The upper portion of the plant was treated-to-wet prior to runoff. The frame of the wiper applicator was constructed with 1.9 cm PVC pipes with two 1.6 cm diameter cotton ropes (approximately 2.5 cm wide and 18 cm in length) affixed to the end of the frame (Figure 1). The glyphosate (Roundup Powermax 3, Bayer Cropscience, St. Louis, MO, USA; 575 g ae L^{-1}) concentrations included were 0 (no glyphosate), 33, 50, and 75%, where the various concentrate dilutions were achieved by mixing glyphosate with distilled water. These concentrations were selected based on the herbicide label (Anonymous 2020). Separate wiper applicators were constructed for each glyphosate concentration tested. The wiper frames were disassembled prior to treatment and the wiper was submerged in a 300 mL solution of the respective concentrations until saturation.

Injury to leafy spurge was estimated 21 days after the 2,4-D treatment (DAT) using a rating scale ranging from 0 to 100%; where 0 equals no injury observed and 100 equals plant death. After the injury evaluations, plants were excised at the surface of the potting media and weighed to collect the fresh biomass. The plant samples were then placed in paper bags and oven-dried at 50 C for 48 hrs. All plant samples were then weighed to collect the dry biomass (g). Pots were maintained as described above for an additional 3 months after 2,4-D treatment (MAT). Shoot regrowth was collected, dried and weighed as described above. After shoot regrowth was collected, pots were not watered for 1 wk to dehydrate the soil. Roots were extracted from the dried potting media and additional potting media was cleaned from the roots via a water rinse. Roots were subsequently dried and weighed as described above. Dry biomass reduction for the treated shoot material (21 DAT), shoot regrowth (3 MAT), and roots (3 MAT) was calculated by dividing the dry biomass of the treated plants by dry biomass of the nontreated plants.

Statistical analysis

Injury estimates and dry biomass reductions were subjected to analysis of variance (ANOVA) using the *Glimmix* procedure in SAS 9.4 (Statistical Analysis Software Institute, Cary, NC, USA) at a significance level of $\alpha = 0.05$. Glyphosate concentration was considered a main effect, while the replications and experimental runs were considered random effects. Replication and experimental run were considered random to allow inferences to be made across broader conditions (Blouin et al. 2011; Moore and Dixon 2015).

Concentration–response curves for injury estimates were fit with a three-parameter log-logistic equation in Sigmaplot 15 (Grafiti LLC, Palo Alto, CA, USA): [1]

$$y = \frac{a}{[1 + \left(\frac{x}{x0}\right)^{b}]}$$

where a is the upper asymptote, x is the glyphosate concentration, x0 equals the effective concentration to cause 50% injury (EC_{50}), and b is the slope at x0.

Glyphosate concentration–response curves for dry biomass reductions of shoot, shoot regrowth, and root were also fit with a three-parameter log-logistic equation [1] in Sigmaplot 15: where a is the upper asymptote, x is the glyphosate concentration, $x0$ equals the GR_{50} (concentration to reduce biomass by 50%]) rate, and b is the slope at $x0$. The GR_{90} (concentration to reduce biomass by 90%) values were derived from the respective equations.

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Results and Discussion

Treated-shoot

Glyphosate concentration did not influence injury estimates (P=0.97) or shoot biomass (P=0.3) of the 2,4-D-treated leafy spurge plants 21 DAT. Injury estimates were approximately 94% for all treatments, and therefore an EC_{50} could not be modeled (Figure 2). All herbicide-treated shoot biomass ranged from 60 to 120% of nontreated plants on average (Figure 3). The GR_{50} value (129%) derived from the model was extrapolated outside of the tested concentrations and not achievable, therefore not reliable (Table 1; Figure 2). The GR_{90} value could not be modeled due to the lack of response (Figure 3). These results suggest that 2,4-D applied alone as broadcast or in combination with wiper-applied glyphosate, does provide greater than 90% injury but does not shoot biomass reduction on leafy spurge within 21 DAT.

Shoot regrowth

Glyphosate concentration influenced shoot regrowth of leafy spurge 3 MAT (P = 0.0012). Leafy spurge shoot regrowth biomass treated only with 2,4-D was approximately 560% of the biomass of nontreated plants (Figure 4). Leafy spurge shoot regrowth was <10% of the biomass of nontreated plants when treated with 2,4-D and when combined with any of the tested wiper-applied glyphosate concentrations (Figure 4). The GR_{50} and GR_{90} values for shoot regrowth were glyphosate concentrations of 7 and 28%, respectively. (Figure 4; Table 1). These results suggest the addition of wiper-applied glyphosate to 2,4-D can significantly reduce t leafy spurge regrowth but there is no difference of biomass reduction between the tested glyphosate concentrations (Figure 5).

Root biomass

Glyphosate concentration influenced the root biomass of treated leafy spurge 3 MAT (P=0.0022). The root biomass of plants treated only with 2,4-D was approximately 160% of the root biomass from nontreated plants. Herbicide-treated leafy spurge root biomass was between 35 to 49% of the root biomass of nontreated plants (Figure 6). The GR_{50} value was a glyphosate concentration of 8%, while a GR_{90} value could not be calculated due to a lack of root biomass reductions (Figure 6; Table 1). The labeled concentrations of glyphosate applied with a wiper

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decreased leafy spurge root biomass by at least 50% compared to roots of nontreated plants (Figure 7).

The results of this experiment indicate that leafy spurge treated with 2,4-D and subsequently with or without wiper-applied glyphosate does incur injury but does not shoot biomass reduction within 21 DAT. However, 3 MAT, shoot and root regrowth were significantly increased when leafy spurge plants were treated with 2,4-D only compared to the other tested treatments. While single applications of 2,4-D are generally not efficacious on leafy spurge, the integration of wiper-applied glyphosate does provide an additional herbicide that is rarely used in pasture/rangeland settings or around sensitive sites for targeted weed management (Gylling and Arnold 1985; Krueger-Mangold). Although 2,4-D + glyphosate broadcast applied is effective on leafy spurge, many land managers may not want to use this mixture due to desirable vegetation injury or death (Gylling and Arnold 1985; Lym 2000). Since the wiper provides a means of selective control with a non-selective herbicide, the leafy spurge plants are managed without injuring or killing desirable vegetation and serves to promote desirable vegetation competition, species richness, and increased land value (Krueger-Mangold et al. 2002; Lamb et al. 2024). Previous research has shown that leafy spurge management increases when desirable vegetation is competitive (Lym and Tober 1997). Since 2,4-D broadcast application followed by the wiper-applied glyphosate reduce leafy spurge shoot and root biomass, this program may be useful in slowing the spread of the infestation. While 2,4-D in addition to wiper-applied glyphosate was effective in this research, caution must be taken to not overuse this tactic. Picloram has been applied extensively and intensively to manage leafy spurge; however, the effectiveness of this herbicide has gradually decreased suggesting resistance evolution (Lym et al. 1996). Other weeds have evolved resistance to glyphosate through recurrent selection (Busi and Powles 2009; Zelaya and Owen 2005). Additionally, when new herbicides are used and applied recurrently, weed community shifts can occur (Culpepper 2006; Hodgskiss et al. 2022). This herbicide program using the combination of both herbicides should reduce selection pressure, but reliance should be avoided (Lake et al. 2023; Renton et al. 2024).

Even though broadcast applications of glyphosate are not effective and can increase vegetative growth, the results from the presented research suggest that the relatively great concentrations of glyphosate applied with a wiper may be more effective. Glyphosate alone

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applied with a wiper should be evaluated to determine why the presented sequential applications were effective. Future research should investigate integrating wiper-applied glyphosate with other effective herbicides (i.e., picloram and imazapic) and non-herbicide tactics (i.e., biocontrol with the leafy spurge flea beetle [*Aphthona* spp.] and mowing). Research should investigate tandem broadcast and wiper applications on one unit to reduce the trips needed to manage weeds. Mixtures of 2,4-D (and related herbicides) and glyphosate applied with a wiper could be utilized in areas where sensitive forbs are desirable. Results from this research suggest that 2,4-D plus wiper-applied glyphosate is effective at reducing leafy spurge regrowth in comparison to 2,4-D applied alone, and further research is to validate these findings under field conditions. Abiotic and edaphic factors influence herbicide activity and plant growth; thus, realized conditions may affect the effectiveness of this herbicide program (Ganie et al. 2017; Hammerton 1967; Moxness and Lym 1989). The long-term above and belowground regrowth should be quantified as well to determine how often a follow-up tactic will need to be implemented. Since leafy spurge can be genetically diverse; this herbicide program should be tested on leafy spurge populations from varying genetically distinct populations and under site-specific production practices (Liu et al. 2023; Rowe et al. 1997).

Practical implications

Leafy spurge is a difficult-to-manage perennial weed species despite extensive efforts to implement effective management tactics. High levels of injury were observed with all herbicide treatments, but short-term (21 DAT) biomass reduction of leafy spurge with any treatment was not evident. Plants treated with broadcast-applied 2,4-D increased biomass compared to nontreated plants 3 MAT. Whereas plants treated broadcast-applied 2,4-D and wiper-applied glyphosate had significant biomass reductions compared to non-treated plants 3 MAT. Since the various concentrations of glyphosate applied with the wiper resulted in similar treated shoot, shoot regrowth, and root biomass reductions, land managers can utilize the lower concentration (33%) which can decrease costs and the amount of herbicide entering the environment. These results also show that leafy spurge treated with 2,4-D-only can result in increased vegetative growth which could exacerbate the spread of infestations. Therefore, providing more evidence that 2,4-D alone is not effective for managing leafy spurge. While 2,4-D, in addition to wiper-applied glyphosate, was effective in this research, caution must be taken not to overuse this tactic.

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Table 1. Parameter estimates from the three-parameter log-logistic equations for biomass of treated-shoots, shoot regrowth, and roots[a].

	Regression parameters[b]					
	a	*x0*	*b*	GR_{50}	GR_{90}	r^2
Shoot	102.3	129.4	1.9	129[c]	NA	0.2
Shoot regrowth	560	6.1	2.6	6	28	0.99
Root	160	7.6	0.5	8	NA	0.99

[a]Abbreviations: GR_{50}, concentration (% diluted concentrate) to reduce biomass by 50%; GR_{90}, concentration to reduce biomass by 90%; NA, not achieved.

[b]*a* is the upper asymptote, x*0* equals the GR_{50}, and *b* is the slope at *x0*.

[c]The GR_{50} value is not achievable and therefore should not be considered reliable.

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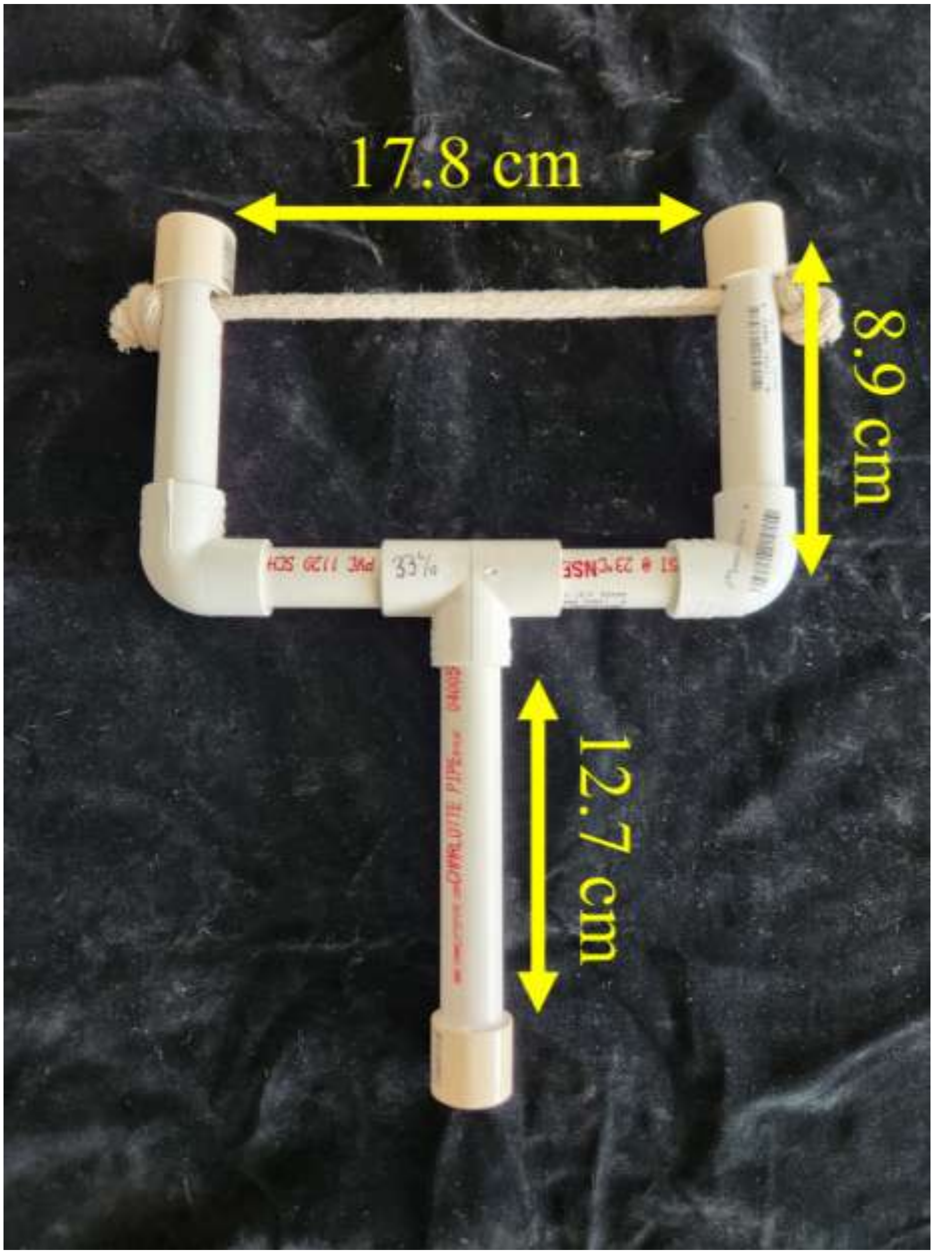

Figure 1. Wiper applicator schematic for the wiper-applied glyphosate experiment.

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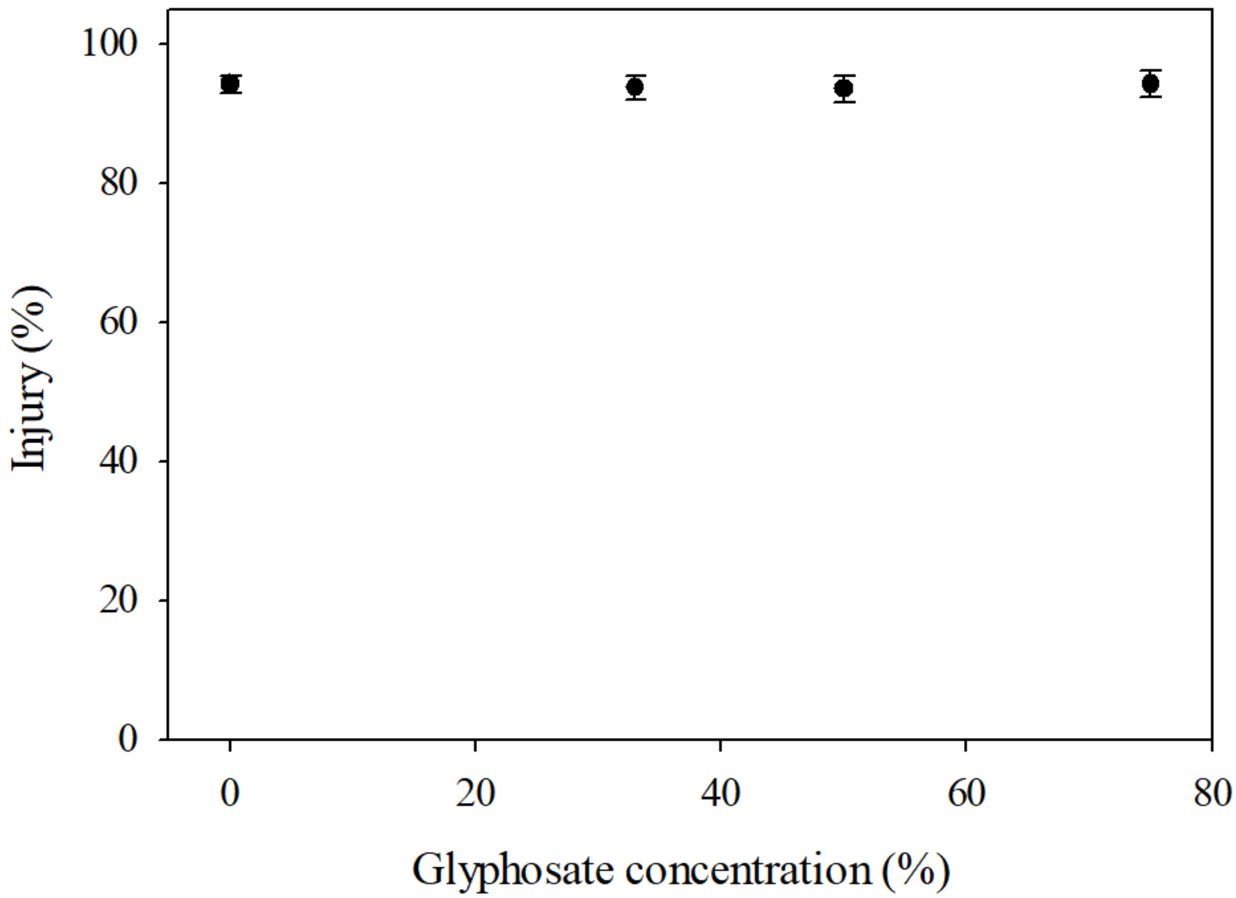

Figure 2. Injury estimates for leafy spurge treated with 2,4-D ester (0%) and the addition of various concentrations of wiper-applied glyphosate. Injury estimates could not be modeled across glyphosate concentrations due to a lack of differential response. The injury estimates of nontreated plants are not included. Error bars represent the standard error of the mean.

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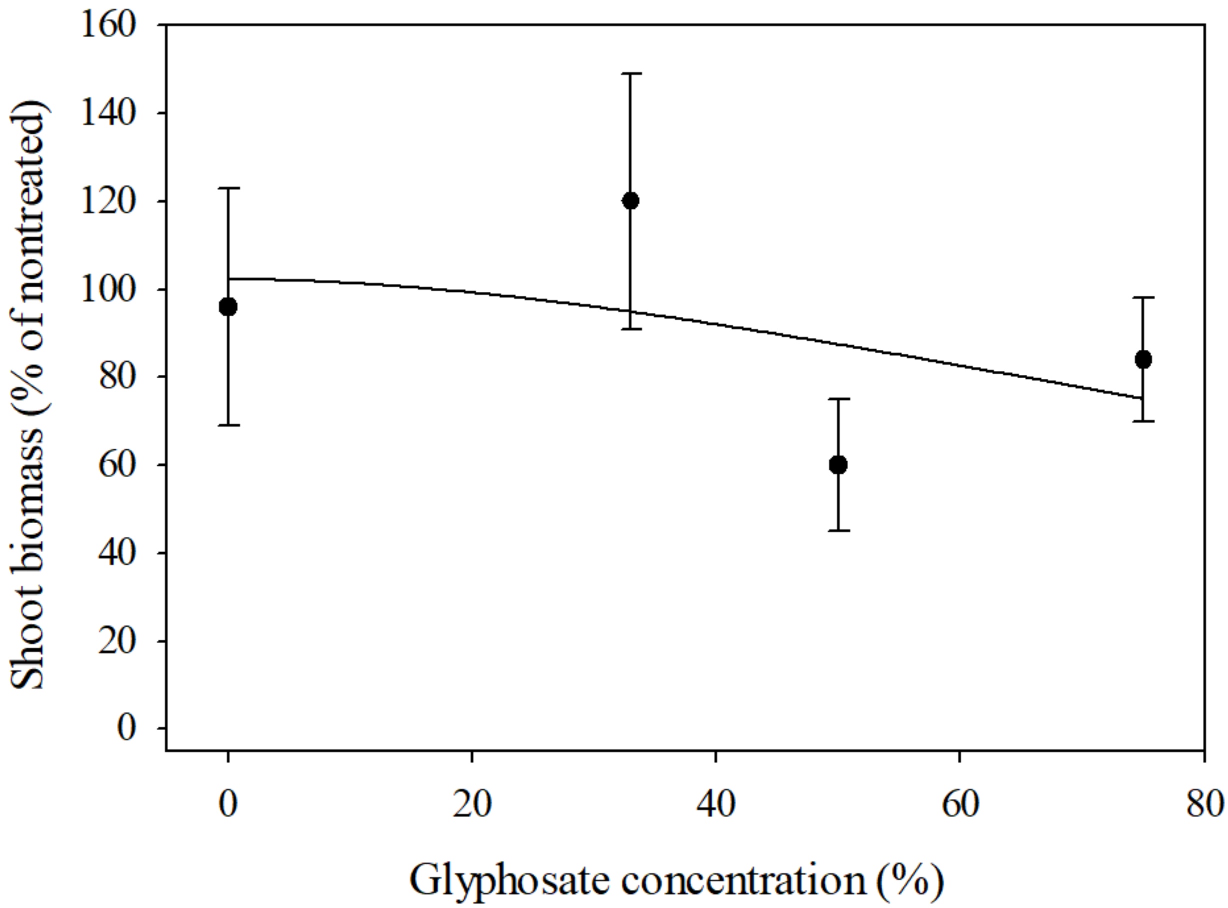

Figure 3. Concentration-response curve fit to a three-parameter log-logistic equation for shoot biomass of leafy spurge treated with 2,4-D and the addition of various concentrations of wiper-applied glyphosate. Error bars represent the standard error of the mean.

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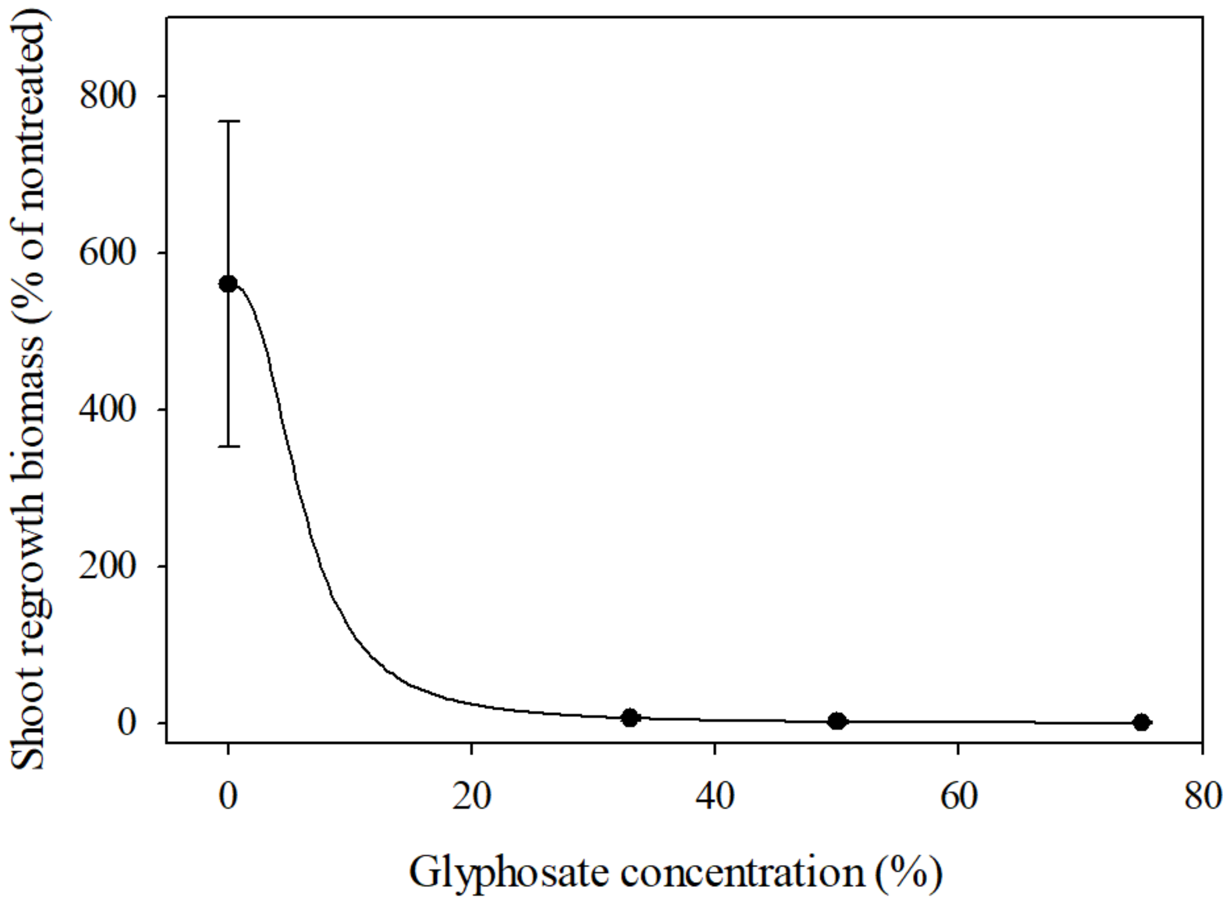

Figure 4. Concentration-response curve fit to a three-parameter log-logistic equation for shoot regrowth biomass of leafy spurge treated with 2,4-D and the addition of various concentrations of wiper-applied glyphosate. Error bars represent the standard error of the mean.

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Figure 5. Visual representation of shoot regrowth of leafy spurge that were nontreated (A), 2,4-D-treated (B), and 2,4-D followed by 33% glyphosate wiper-applied (C). 2,4-D followed by 50 and 75% glyphosate wiper-applied are not shown as no regrowth occurred.

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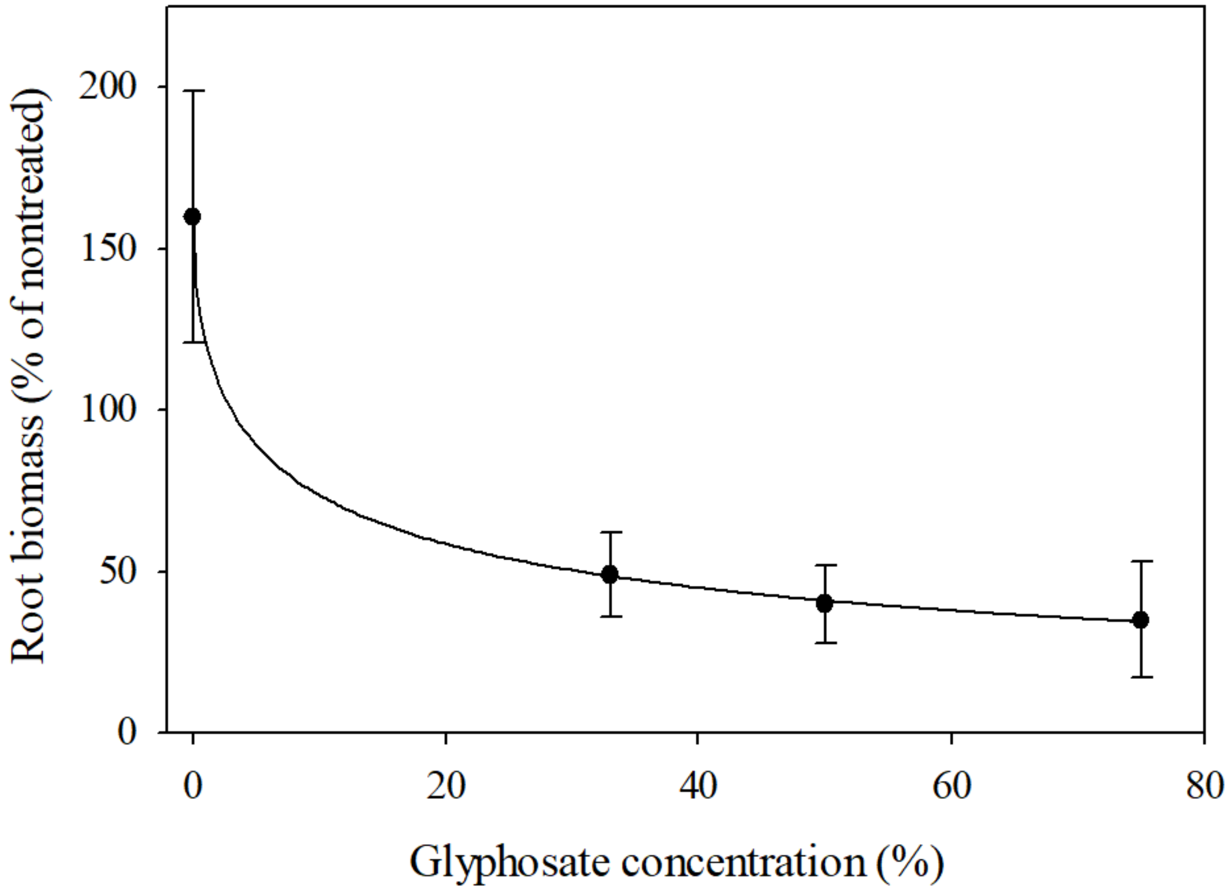

Figure 6. Concentration-response curve fit to a three-parameter log-logistic equation for root biomass of leafy spurge treated with 2,4-D and the addition of various concentrations of wiper-applied glyphosate. Error bars represent the standard error of the mean.

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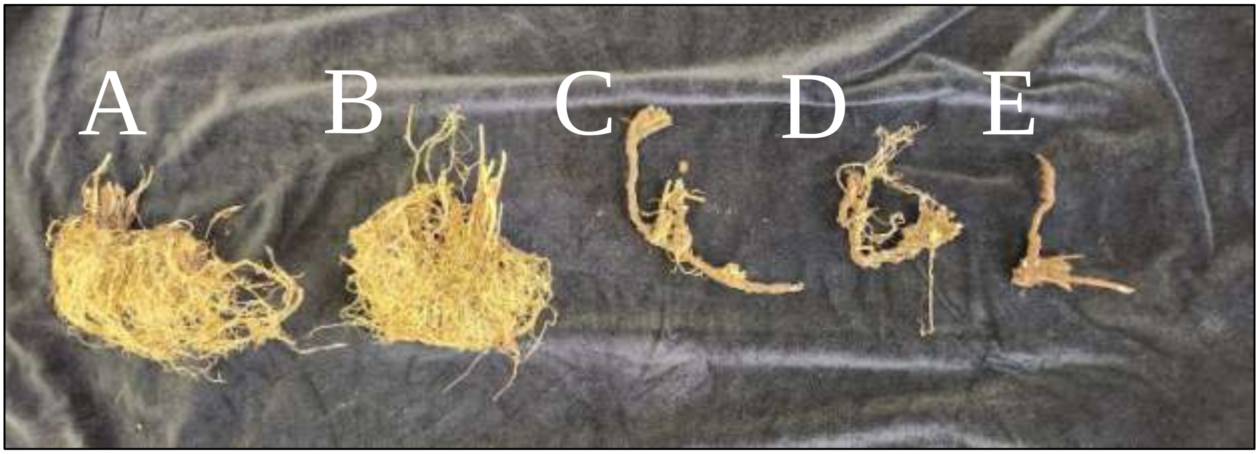

Figure 7. Visual representation of root biomass of leafy spurge that were nontreated (A), 2,4-D-treated (B), 2,4-D followed by 33% (C), 50% (D), and 75% (E) glyphosate wiper-applied.